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November 14, 1946.

Dear Sol:

Thanks very much for your letter; my first question(on the basis of the effect of exogenous N) was not well founded and I think I have it cleared up, but I would put it as follows: $Pr \underset{\overleftarrow{k_1}}{\overset{\vec{k_1}}{\rightleftarrows}} E_1$; $-\frac{\partial \overleftarrow{k_1}}{\partial S_1} \gg 0$ The absence of exogenous N affects the overall rate of this reaction by decreasing (Pr), and the forward speed of 1). Particularly in the presence of S_2 which would allow E_2 to accumulate at the expense of Pr. O.K.

As to the azide effect, the stumbling block in my mind was that $\overleftarrow{k}$ should be affected. I guess that will have to be taken as the empirical fact, butdo you postulate that this reaction requires the same transfer of energy as the forward reaction? It might be better to provide a different sink for E_1 in the absence of substrate. Is it possible to decrease $\vec{k}_1$ without affecting $\overleftarrow{k}$?

I hardly know whether to call 'crossing' a verbal or scientific advance. Hybridization is the only mechanism that can reasonably explain all the facts presented (and some others), so I would regard the prototrophs in a mixed culture of mutants as being the results of a 'cross' They are quite rare,however; I don't quite understand what experiment Hershey and you tried: was it to grow a fermenter and non-fermenter together and examine the fermenters for their stability in the absence of the substrate?? The experiment I mentioned to you last letter has been tried; the character of lactose fermentation segregates very nicley however

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in spite of the fact that lactose was the only carbohydrate present throughxxxxx out the cultivation and mixed culture and plating of the bugs. However peptone was present in the culture medium, and asparagine in the plating medium; I am going to repeat the whole thing on synthetic medium with lactose as the only source of carbon and look for unstable fermenters. In this xxxxxxxx experiment, T-L-B_1-T_1^s-Lac- and B-M-T_1^r-Lac+ were grown separately in nutrient broth plus 1% lactose, and then incubated together in this medium for two hours. The cells were washed and plated in minimal (lactose) medium to recover the prototrophs. The following types were found:

	T_1^r Lac-	T_1^s Lac+	T_1^r Lac+	T_1^p Lac+
T+L+B_1+B+M+ :	31	6	16	4

The excess of T_1^R over T_1^s in such crosses is a consistent trend + probably due to linkage

T_1 refers to resistance to phage; the other characters are nutritional requirements. A few Lac+ were tested for stability of fermentative character after growth on lactose-deficient medium. No change. While this suggests that there does not exist a mechanism here as in yeast, it deserves further study; it could reflect different dependence of the plasmagene on the gene, which might also be determined by studies on the kinetics of adaptation. On the other hand, the mutated enzyme(??) might be equally well stabilized by the substrate, allowing segregation of the gene. Such a phenomenon could be picked up in yeast by an examination of the effect of substrates which are not fermented on the rate of de-adaptation to a different substrate. We have been hearing rumors that you have transformed a raffinose-non-adapter to a raffinose-adapter. Is that right??

Regards.

Sincerely
Josh.